DYNAMICS MODELS OF VIRUS/CELL TURNOVER IN HIV INFECTIONS

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Introduction

HIV replicates in human cells expressing CD4 and coreceptor molecules, predominantly activated CD4 T cells and macrophages, and like other virus infections causes global activation of the immune system. The dynamics of HIV infections can be divided into two major phases: acute (non-steady) and quasi-steady. During the acute phase the virus rapidly disseminates until most of the susceptible target cells are infected; the immune system activation during this period further accelerates the infection by providing additional target cells (activated CD4 T cells). The exponentially increasing self-accelerating virus infection leads to rapid decline of the number of CD4 T cells in the blood by direct and/or indirect cytopathic effects and redistribution to lymphoid tissue. Subsequently, the number of virus-producing cells decline and CD4 T cell counts in the blood increase to levels close to normal due to immune responses, and other mechanisms including exhaustion of susceptible target cells, redistribution and homeostatic regulation. This is the end of the acute phase, which typically develops within one to three months.

The quasi-steady state of HIV infections is characterized by an almost perfect balance between the production and clearance of both virus and CD4 T cells for a prolonged period of time (years). It can be further subdivided in many cases into two stages (slow and rapid) characterized by relatively slow and rapid decline of the number of CD4 T cells, respectively. The decline of the CD4 T cell counts correlates with an increase in the virus concentration and leads to the development of opportunistic infections defining AIDS (at counts below 100-200 cells per μl). The specific mechanisms by which the virus causes the decline of the CD4 T cells and pathogenesis are currently unknown in spite of the large amount of work and many hypotheses. The quantitative analysis of HIV/cell dynamics by a combination of experimental and mathematical methodologies may not only help in the elucidation of the mechanisms of HIV immunopathogenesis but also may have major implications for optimization of treatment and development of vaccines.

During the early stages of the acute phase of HIV infection when the specific immune response is still developing, the rates of virus dissemination may reflect some of the virus/host interactions which are important for pathogenesis. Therefore we have hypothesized that by measuring the initial rate constants one can quantify the virus/cell interactions as an integral measure of the virus infectivity and cell susceptibility.

To test this and other hypotheses we have been studying systems of increasing complexity. The simplest system is cultured cells where most of the parameters, e.g. the number of uninfected and dead cells, can be accurately measured under controlled environment. The more complex system is simian immunodefficiency virus (SIV) and chimeric SIV/HIV (SHIV) infections of monkeys where one can precisely define the onset of infection and the initial dose of infectious virus. The initial dynamics of HIV infections in humans is most difficult to analyze for lack of knowledge of the exact time of infection onset and initial dose. However, several recent studies of patients under highly active antiretroviral therapy (HAART) who discontinued therapy offered the unique opportunity to study the virus dynamics at relatively well defined initial conditions.

Infection Dynamics in Cultured Cells

About a decade ago we have been studying HIV infections of tissue cultures to find out which mode of HIV transmission is the most effective, how many infectious virus particles are produced by one infected cells and what is the relationship between the virus and cell dynamics (Dimitrov D.S. et al., 1993). We used a standard culture system where the cells are at the bottom of the flask and therefore their local concentration is much higher than the average bulk concentration; this system imitates to some extent the conditions in the lymphoid tissue where the CD4 T cell concentration is about two orders of magnitude higher than the average concentration in a typical tissue culture

(5×10^5 cells per ml). In addition, this is relatively well mixed system because periodically (every 2-3 days) the cells are split to keep their concentration constant and during splitting they are mixed; also they move and aggregate. In a typical dynamic pattern the virus concentration increased exponentially in the supernatant, the uninfected cells declined and the number of dead cells (which is proportional to the number of infected cells but delayed) exhibited a maximum (Fig. 1). The exponential pattern was independent on whether infected cells or cell-free virus was used to initiate the infection. If the amount of cell-free virus was derived from the same number of infected cells, the infection initiated by virus derived

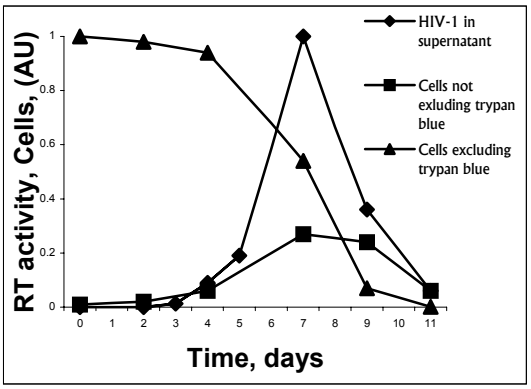

Fig. 1. HIV-1 and cell dynamics in tissue culture infections

from 10^4 cells was equivalent to infection initiated by one infected cells suggesting dominance of cell-to-cell transmission. In addition, the half-life of cell-free infectious virus was relatively short (on the order of hours) under the conditions of tissue cultures (Dimitrov D.S. et al., 1992). Thus, we consider only the dynamics of infected cells and assume that the virus concentration is proportional to their number.

When the infection was initiated by different amounts of virus (serially diluted 10-fold, 100-fold, etc.) we found two relationships: first, the virus concentration increases exponentially with slopes in logarithmic scale essentially the same for every dilution, and second, the time required to reach the infection peak (t_P) or any other level of viremia is proportional to the logarithm of the initial virus dose dilution (D). These two relationships were described by a simple empirical formula that was able to fit the experimental data for a number of systems and define an important parameter – infection rate constant (k):

$$k = \left(t_P - t_P^*\right)^{-1} \ln(D),$$

where t_P^* corresponds to the time of peak at $D = 1$. We found that the infection rate constant k varies with the virus strain and cell type but is not dependent on the initial dose of virus used to initiate the infection. The highest k was obtained for activated primary cells.

We tried to understand the biological aspects of k and developed two types of models: first, a very simple model of virus spread by subsequent cycles of infection assuming that infected cells release all virus at the end of the infectious cycle and die instantaneously which is similar to what was observed with the burst of bacteria caused by bacteriophages. In this case by using simple mathematics we found that k is proportional to the logarithm of the burst size (basic reproductive ratio) and inversely proportional to the time required to complete one cycle of infection. The analysis of the experimental data suggested that the burst size is on the order of several to several hundred in dependence on the particular virus/cell system.

To describe the complete virus cell dynamics (not only the initial stages) in cultured cells we used a model based on a system of differential equations (Spouge J.L. et al., 1996):

$$dC/dt = -k_i CI + k_c C\left(1 - \frac{C+I}{C_m}\right),$$

$$dI/dt = k_i CI - k_d I\ ,$$

where: C is concentration of uninfected susceptible cells; I - concentration of infected cells; C_m, k_i, k_c, and k_d are constants characterizing the carrying capacity of the cultured cells, the specific infectivity of the virus/cell system, the effective growth rate of uninfected cells and the rate of death of the infected cells, respectively.

This model not only described the dynamic pattern depicted on Fig. 1 but also predicted

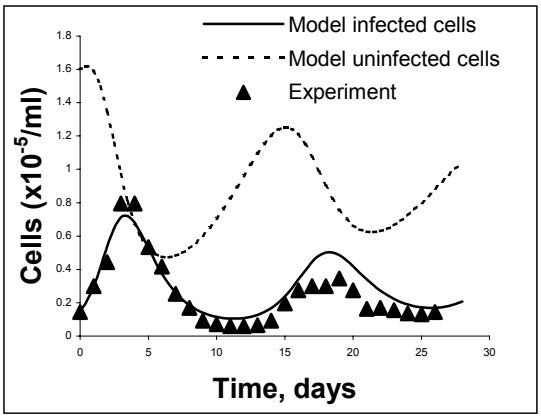

Fig. 2. HIV-1 spread in tissue cultures

oscillations. Typically oscillations are not observed in tissue cultures because the number of cells is kept constant by splitting the cultures (k_c = 0); however, under conditions where cultures are not split oscillations do exist (G. Englund, M. Martin and D.S. Dimitrov, unpublished). The experimental data were fitted with the model at reasonable values of the parameters (Fig. 2). In this case the infection rate constant k can be expressed by the specific infectivity

constant k_i, the initial cell concentration C_0 and the death rate constant k_d: $k = k_i C_0 - k_d$.

The basic conclusions from our experiments and models of cultured cells are: 1) the dynamics of acute HIV-1 infections in tissue cultures can be quantitatively described by mathematical models. Predator-prey models predict oscillations that were observed experimentally; 2) the infection rate constant, characterizing the early infection dynamics, depends on virus infectivity and pathogenicity, local (but not average) cell concentration and susceptibility; it does not depend on the initial infectious virus dose; 3) the infection rate constants can be quantified by measuring times required to reach certain level of viremia at different virus dilutions or the slope of the initial rise in virus concentration. 4) The burst size varies significantly for different virus/cell systems. 5) The infection rate constants measured in cultured cells could be used to characterize the initial dynamics of virus/host cell interactions, and effects of drugs and vaccines.

Dynamics of Acute Infections in Monkeys

Patterns of HIV/cell dynamics similar to those in cultured cells were observed in SHIV or SIV infections of monkeys: an initial exponential increase of the virus concentration followed by decline, rise and decline of the number of cells containing SHIV proviral DNA, and continuous decrease of the CD4 T cell concentration (Fig. 3) (Endo Y. et al., 2000). Tittering

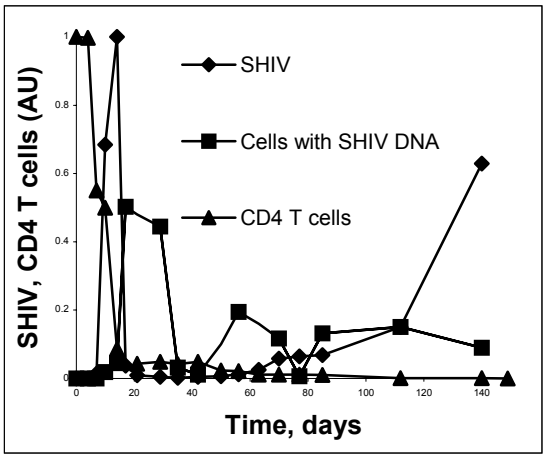

Fig. 3. SHIV and cell dynamics in macaques

of SHIV infections in macaques can be described by the same simple relationships as in tissue cultures. Therefore as in tissue cultures one can determine k from two different relationships: the exponential rise in the virus concentration and the average k from tittering the virus.

After this initial period of several weeks in some cases the virus concentration increases and the CD4 T cell counts remain low – typically this leads to immunodefficiency and death within months or years. In other situations the virus concentration remains low and the CD4 T cell concentration increases – in this case the monkeys remain healthy for years. Whether the infection will be pathogenic or not depends on the virus strain, the initial dose of infectious virus, route of infection, and the host, in particular its immune system. We and others have hypothesized that the initial race between the virus dissemination and the development of immune response could determine to some extent the clinical outcome. Indeed, for macaques infected with SHIV we found a trend that higher k correlate with the logarithm of HIV concentrations at 3 months and disease (Spearman correlation, $R = 0.87$ and $P = 0.058$; $n = 5$). The effective rate decline constants, d, characterizing virus decay during the first two weeks after the infection peak were statistically significantly correlated to clinical outcome ($R = 0.78$ and $P = 0.01$; $n = 9$); higher rate constants corresponded to greater survival probably due to more effective immune responses.

We also analyzed two groups of macaques infected with low and high doses of the pathogenic SIVmac239 where data were collected every day for the first 8 days (J. Liffson, unpublished, data not shown). Again at the two different infectious doses the slopes were about the same but the curves were shifted; by using the equations relating these shifts with the infectious dose we derived average k which were about the same as those derived from the slopes for individual macaques. Therefore the initial dose does not affect k and also does not affect the level of viremia at the infection peak but the time required to reach it.

The variation between individual macaques was reflected in the differences in k. As in the case of macaques infected with SHIV in the case of SIV infected macaques the infection rate constants were correlated with the logarithm of SIV at 3 months and clinical outcome; although the number of monkeys was small (eight) the correlation was statistically significant. The differences in k were not large; however because the rise in viremia is exponential even small differences could lead to significant changes; e.g. if k_1=1.8 and k_2=1.0 then at each cycle the ratio is about 2 which after 10 cycles will lead to an impressive difference of 1000.

The basic conclusions from the mathematical analysis of acute infections in monkeys are: 1) initial dynamics of acute SHIV/SIV infections resembles HIV-1 infections dynamics in tissue cultures; 2) rate constants of primary SHIV/SIV infections can be accurately quantified; 3) The infection rate constants characterizing the very early stages of primary SHIV/SIV infections tend to correlate with clinical outcome; 4) the rate constants characterizing the virus decline after the infection peak correlate with clinical outcome; 5) one can speculate that the initial race

between virus infection and immune responses is an important determinant of immunopathogenesis.

Infection Dynamics in Patients off HAART

The dynamics of HIV in patients off HAART somewhat resembles that in cultured cells and in monkeys – the virus concentrations rises exponentially

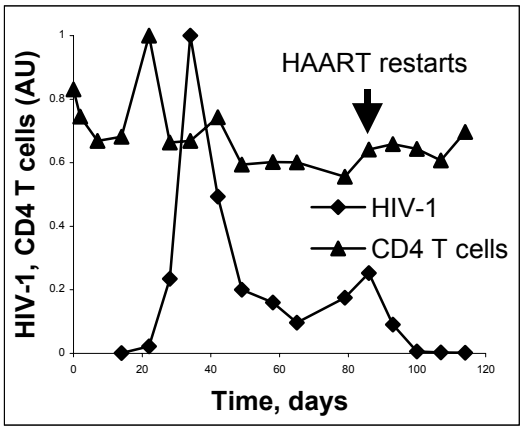

Fig. 4. HIV-1 and cell dynamics in patients off HAART

after the therapy is interrupted and then can reach a quasi-steady state or decline and oscillate (Davey R.T. et al., 1999) (Fig. 4). Simultaneously with the increase in the virus concentration the number of the CD4 T cells begins to decline and can also oscillate. We found that k is on average about 2-3-fold smaller then k for cultured cells or in acute infections in macaques. The lower rates of initial virus dissemination could be due to the suppressive effects of an already developed specific immune response, lower number of target cells, lower susceptibility to infection, and/or other reasons.

With the increase of the virus concentration the number of dividing cells as measured by BrdU incorporation was rising, although at slower rate, and on average was about 10-fold higher at the infection peak compared to the level under HAART. This raises the interesting possibility that during the infection the number of susceptible cells is increasing thus leading to an effect of self-acceleration. However, there is no direct experimental evidence for this possibility. Importantly, there was no correlation between parameters characterizing HIV dynamics (time to reach infection peak, k) and the number of latently infected cells suggesting the existence of other reservoirs or/and a small reservoir of actively replicating virus.

The basic conclusions from our analysis of HIV dynamics in patients who interrupted their treatment are: 1) the infection rate constants for patients off therapy are significantly lower than those for primary infections possibly due to immune responses or/and other causes; 2) the infection rate constants do not correlate with levels of viremia at or after the infection peaks, and the capability of controlling viral replication after HAART discontinuation; 3) the infection rate constants correlate to some extent with the rates of increases in the percentage of proliferating CD4 T cells but not with the rates for CD8 T cells suggesting the possibility for self-accelerating infections; 4) HIV-1 dynamics after HAART interruption does not correlate with the number of latently infected CD4 T cells during HAART suggesting the existence of other reservoirs or/and a small reservoir of actively replicating virus.

Quasi-Steady State

During the quasi-steady state the virus/cell turnover is high although the virus and cell concentrations do not change significantly with time (Ho D.D. et al., 1995; Wei X. et al., 1995). Due to the high turnover antiretroviral treatment of patients with HIV leads to rapid decline of the virus concentration. We found that the rate constants of the very initial (first week on therapy) virus decay correlate with virus concentrations at three months on therapy (that correlates with clinical outcome) and can be used as a very early predictor of drug efficacy (Mueller B.U. et al., 1998). The dependence of the virus decay constants on drug efficacy suggested that even under HAART HIV continues to disseminate (Grossman Z. et al., 1999a).

Antiretroviral treatment leads also to an increase in the blood CD4 T counts. We and others suggested that the very initial increase is due to redistribution from the lymphoid tissue to the blood (Mosier D.E, 1995; Sprent J. and Tough D., 1995; Dimitrov D.S. and Martin M.A., 1995). HAART also decreases the number of dividing cells as measured by BrdU incorporation *ex vivo* (Lempicki R.A. et al., 2000). The diminished cell turnover is probably due to the decline in the number of cells activated by HIV. We proposed that the rapid cell turnover induced by HIV is due to a subpopulation of immune activated cells; at the same time a subpopulation of slowly dividing cells ensuring the regeneration of the immune system may or may not be affected significantly by HIV (Grossman Z. et al., 1999b). The mathematical model based on this concept described well experimental data for cell turnover in monkeys infected with SIV. Overall, it appears that HIV induces a generalized activation of the immune system leading to high turnover of the immune system cells; how this is related to the slow decline of the number of CD4 T cells remains unknown.

Major Conclusions

A critical parameter characterizing the initial infection dynamics is the infection rate constant; it depends on virus infectivity and pathogenicity, and cell susceptibility and concentration, but not on the multiplicity of infection.

HIV-1 infection dynamics in tissue cultures resembles acute SHIV/SIV dynamics in the absence of immune responses.

Initial infection rate constants could be predictive of clinical outcome.

The infection rate constants for HIV-1 infected patients off HAART are significantly lower than those for primary infections possibly due to immune responses or/and other causes.

Quantification of infection rate constants may help in characterizing the pathogenicity of primary HIV-1 isolates and vaccine efficacy.

The analysis of HIV dynamics in patients off HAART suggests the existence of small reservoir of actively replicating virus that has major implications for treatment strategies.

The rate constants of the initial virus decline correlate with clinical outcome, and can be used as an early predictor of drug efficacy and for optimization of treatment.

HIV induces generalized activation of the immune system and high turnover of immune system cells but how this is related to the slow decline of CD4 T cells and immunodefficiency remains unknown.

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